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Role of dialysable solutes in the mediation of uremic encephalopathy in the rat

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Role of dialysable solutes in the mediation of uremic encephalopathy in the rat. This study addresses mechanisms of the clinical, encephalopathic uremic illness and its suppression by dialysis. Renoprival rats were treated with peritoneal dialysis (8 exchanges per day, 30 min dwell), or untreated (attrition group), and their EEG's were automatically sampled overnight and subjected to power spectrum analysis as an index of encephalopathy. As in man the background rhythm of the quantified EEG (Q.EEG) in the attrition group slowed with time as extracellular fluid composition became increasingly abnormal; these changes were normalized by therapeutic dialysis (TD) using standard, commercial dialysate. However, Q.EEG slowing was only partially normalized by solute-specific dialysis using "mock uremic dialysate" (M-UD), prepared from laboratory chemicals to equal plasma concentrations in preterminal uremic rats of urea, creatinine, potassium, phosphorus, calcium, magnesium, bicarbonate, sodium, and chloride. When only phosphate was added to TD, the Q.EEG slowed to the same level achieved after M-UD. We conclude that uremic encephalopathy in this model is produced by an unknown neurotoxin and augmented by one or more of the M-UD solutes, phosphate being a likely candidate. To localize the encephalopathic effect, regional brain glucose uptake was estimated in 20 discrete brain areas. Significance of reduced uptake in three areas is discussed.

These studies address two fundamental, unanswered questions: (1) How does renal failure make patients sick? and (2) How does dialysis reverse that clinical illness? While the significant success of dialysis procedures makes these questions less clinically urgent, both the empirically described illness ("clinical uremia") and its response to empirical treatment (dialysis) deserve a rational, mechanistic explanation.

The clinical illness of severe uremia is most cogently typified as an encephalopathy, an "illness behavior" represented by derangement of psychic function (affect, cognition and, terminally, vegetative function) at all levels of cerebral organization [1–7]. In both animals and man, this syndrome of uremic encephalopathy is indexed by certain neurobehavioral measures [3] and by reproducible and characteristic changes in the background rhythm of the quantitative electroencephalogram (Q.EEG). These have been described as "slowing", or the loss of high frequency activity [8–13]. The syndrome and its electroencephalographic derangements are ameliorated by both peritoneal and hemodialysis [3, 14]. From this we infer that the proximate neurotoxic factor is either itself dialysable (the dialysable neurotoxin hypothesis of Schreiner and Maher [15]) or that its effects indirectly respond to dialytic removal of some other solute (the "trade-off" hypothesis of Bricker [16]). Clinically, the degree of uremia is also indexed by the extent of derangement of the levels of certain commonly measured extracellular fluid (ECF) solutes, notably urea, creatinine, calcium, phosphorus, potassium and bicarbonate. These levels change due to the uremic patient's reduced renal excretory capacity and, together with the symptomatology of uremic encephalopathy, their levels are restored toward normal by dialysis. The extent to which any of these moieties contribute to uremic encephalopathy is, however, unknown.

The pathogenesis of uremic encephalopathy has been explored by a number of authors in terms of the electrical and metabolic indices of brain function. In the rat, Van den Noort et al [17] found that creatinine, phosphate, ATP and glucose content were increased but that there were corresponding decreases in creatinine, AMP, ADP and lactate. Total brain adenine nucleotide content was normal to low. The work of Mahoney, Sarnacki and Arieff [18] on the uremic dog would suggest that energy charge in the uremic brain is unchanged while substrate utilization is reduced.

The present investigation sought to examine the encephalopathic effect of uremia in a nephrectomized rat model amenable to chronic conscious ambulatory Q.EEG recording and peritoneal dialysis (PD). Our purpose was to investigate the correlation, if any, between encephalopathic changes in the EEG and the abnormal concentration of commonly measured ECF solutes that occur in renal failure and are modified by dialysis. In addition, because glucose uptake and metabolism is critical for brain function we postulated that the observed neurobehavioral deficits and their amelioration by dialysis should be reflected in differences in quantitative regional cerebral glucose uptake (rCGlu). With this model, the "dialysable neurotoxin" hypothesis was tested insofar as it relates to the combined influences of urea, creatinine, calcium, phosphorus and potassium, while sodium and chloride and presumably bicarbonate remained essentially unchanged. The method has previously been successfully used to maintain conscious ambulatory renoprival animals for up to 13.5 days and to determine the relationship between degree of uremia and degree of Q.EEG slowing in this species [14, 19]. It was hypothesized that dialysate formulated to contain "uremic" levels of these solutes—so as to prevent

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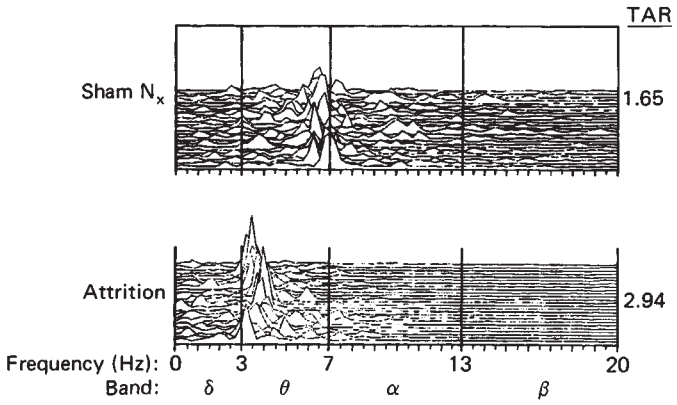

Fig. 1. *Typical compressed spectral arrays of the Q.EEG measured from a control, sham nephrectomized (Sham Nx, upper frame) and a uremic, nephrectomized (Attrition, lower frame) animal.* The frequency boundaries (Hz) are shown for the delta, theta, alpha and beta bands. Ordinate indicates power. The arrays are composed of 25 four second epochs. The figure illustrates the phenomenon whereby the theta:alpha ratio (TAR) increases as the power distribution shifts to the lower frequencies; that is, as the background rhythm slows.

their dialytic removal from the renoprival rat's extracellular fluid (ECF)—would maintain or exacerbate the encephalopathic syndrome if one or more of them contributed to the neurotoxic effect. Finally, the model was employed in a single, initial example of single- solute-specific dialysis in which Q.EEG responses were recorded when dialysate levels of phosphate only were elevated to "uremic" levels.

Methods

Animals

There were fifty-five male Sprague-Dawley rats (Sasco Laboratories, 250 g) used in these studies. Animal housing and experimental facilities employed identical temperature and humidity control with 12 hour light-dark rhythm. Animals were acclimated for one week prior to surgical implantation of chronic extradural silver ball EEG electrodes. Electrode implantation was performed using the method of Lipman and Harshman [20]. Briefly, monopolar electrodes were chronically emplaced through burr holes in the skull bilaterally over frontal and occipitoparietal cortices, a fifth, reference, electrode being embedded in the bone of the nasion. The electrodes were connected to a plug permanently mounted to the animal's skull. Fourteen days were permitted to elapse to ensure proper healing. Quantitative electroencephalographic (Q.EEG) measurements were then made, sampling for 100 seconds every two hours from groups of ten rats at a time for one week, using an automated procedure we have previously described [21, 22]. Slowing of the dominant frequencies due to encephalopathy was therefore indexed in these Q.EEG measurements by deriving the theta:alpha ratio (TAR) which is the ratio of powers (μV^2) in theta and alpha bands (Power 3 to 7 Hz/Power 7 to 13 Hz; Fig. 1). Eight 100-second Q.EEG measurements were obtained each night, the TAR was derived from each measurement and these values averaged over the entire measuring period (17:00 hr to 07:00 hr) for each rat. A minimum of three nights of Q.EEG measurement were chosen from those obtained during the control, pre-nephrectomy, stage of the study, and the average TAR values were then computed over these three nights for each animal.

The animals were singly caged in clear acrylic observation chambers and their electrodes attached by light, flexible shielded cables to a digital switch box which sequentially selected the individual rat whose signal was to be measured by the EEG machine, from the group of ten connected. Selection was made by a software generated control word sent from the parallel port of a microcomputer (IBM PC/XT) which also received and processed the signal. The selected rat's EEG signal passed through the switch box to the EEG machine producing a paper record. The amplified signal was additionally sent to an analog-to-digital converter (Tecmar DT5712) where the digitized signal was read into the IBM PC/XT computer, and stored on the hard disk of the system. The system was also equipped with a digital tape backup unit (Irwin Magnetics) for permanent archiving of data. Following an EEG recording session the data were displayed on a monochrome monitor and, where required, a hard copy was produced on the attached dot-matrix printer (Epson). Data management was accomplished on a remote mainframe computer (VU VAX 8800) accessed by modem.

Surgical

After control Q.EEG recordings had been made, all animals were surgically prepared for dialysis. The method of surgical implantation of the peritoneal dialysis catheter guide is described more fully elsewhere [23]. In use, a sterile peritoneal dialysis (PD) catheter was inserted through a chronically implanted guide to rest intraperitoneally. Through this catheter sterile dialysis fluid was instilled and withdrawn. In our preliminary studies we found that a 60 minute equilibration with therapeutic dialysate (Table 2) gave equilibrated peritoneal dialysate solute (EPD solute) concentrations which approximate those measured from the uremic rat's serum (Table 1). Such measurements, therefore, were made twice daily at 07:00 hours and 14:00 hours in lieu of blood sampling. Dialysate volumes were restricted to 20 ml to minimize solute removal from body stores. In those animals subjected to PD for control of uremia, eight exchanges (each of 30 min dwell time) were conducted per day per rat, using 30 to 35 ml volumes (7.5% of body weight [14, 24]). At the end of the dwell time, dialysate urea levels were 75% equilibrated with serum. Bilateral nephrectomy in those animals rendered uremic was performed using a ventral approach, with omentectomy to prevent occlusion of the PD catheter by omentum. Surgically prepared animals were then assigned to the following groups and treated according to the protocol illustrated in Figure 2.

Treatment groups

Animals of the *Attrition Group* ($N = 10$) were subjected to jugular vein catheter implantation, bilateral nephrectomy and omentectomy. These animals were not dialyzed except insofar as EPD samples were taken twice daily. The *Therapeutic Dialysis Group* (TD, $N = 25$), was treated as above except that eight exchanges of peritoneal dialysis (30 ml vol, 30 min dwell time) were conducted using a commercial therapeutic dialysate (Table 2) between 08:00 hours and 14:00 hours each day, commencing 24 hours after nephrectomy. The *Mock-Uremic*

Table 1. Equivalence of serum and equilibrated peritoneal dialysate chemistries in nephrectomized animals (N = 10)

Sample	Urea	Cr	Ca	P_i	Na	K	Cl	CO_2
	mg/dl			*mEq/liter*				*mM/liter*
Blood	262	4.45	8.83	16.4	134	10.87	97.5	10.0
Dialysate	241	4.15	8.3	13.5	131	10.17	96.5	10.5
Ratio: $\frac{\text{Dialysate}}{\text{Blood}}$	0.91	0.91	0.94	0.83	0.97	0.99	0.99	1.0

Table 2. Solute compositions of therapeutic and special dialysate solutions; Mock uremic dialysate (M-UD), containing several solutes at uremic concentration and phosphate-UD (P-UD) containing only phosphorus at uremic concentration

Solute *Concentration*	PD fluid		
	Therapeutic dialysate[a]	Uremic dialysates	
		M-UD	P-UD
Na *mEq/liter*	132	131	131
K *mEq/liter*	0	8.5	0
P_i *mg/dl*	0	15.9	15.1
Ca *mg/dl*	7.0	8.8	7.3
Mg *mEq/liter*	1.5	2.38	1.5
Cl *mEq/liter*	102	100	102
HCO_3 *mEq/liter*	0	11.63	0
Lactate *mEq/liter*	35	0	35
Gluconate *mg/dl*	0	2.65	0
Creatinine *mg/dl*	0	3.7	0
Urea *mg/dl*	0	262	0
Glucose *mg/dl*	1500	922	1500

[a] Inpersol 1.5%
[b] Urea nitrogen = [Urea] × 28/60

Dialysis Group (M-UD, N = 10) was treated as above, except that the dialysate was formulated to contain concentrations of urea, creatinine, calcium, phosphorus, sodium, potassium, chloride, and bicarbonate similar to those found in the blood of post-48 hour nephrectomized rats (Tables 1 and 2). The *Phosphorus Dialysis Group* (P-UD, N = 8) was treated as above, except that the dialysate contained uremic (elevated) levels of phosphate, but was otherwise identical to therapeutic dialysate. The *Sham Nephrectomized Group* (Sham, N = 16) was omentectomized but not nephrectomized, and these renally intact rats were dialyzed as above, commencing 24 hours after nephrectomy. As illustrated in Figure 2, daily EPD sampling, dialysis, and overnight Q.EEG measurement was continued for 70 hours.

Regional cerebral glucose uptake

This was studied in animals of the Sham, Attrition and TD groups. After the morning EPD sample of the fourth day, the conscious unrestrained animals were administered a 100 μCi pulse of ^{14}C-2-Deoxy-D-glucose via the jugular venous catheter and 45 minutes later sacrificed under light halothane anesthesia by exsanguination, perfusion and fixation (100 ml 0.9% NaCl followed by 300 ml phosphate buffered formalin, 10%, pH 7.2). Perfused brains were harvested, fixed for five further days and then microdissected for ^{14}C-2DG estimation. The microdissection technique has been published elsewhere (Lipman and Tolchard, *Life Science*, in press).

Briefly, this was accomplished by placing the fixed brain ventral side uppermost in a stainless steel template (Activational Systems, Inc.) and slicing coronally at 1.0 mm intervals. The tissue slices were laid on a rubber cutting mat and 25 regions of interest extirpated by appropriately sized tissue punches using the method of Palkovits and Brownstein, and Paxinos and Watson [25, 26]. The wet tissue samples were weighed, placed in polyethylene cryovials and vacuum desiccated over calcium carbonate for 36 hours. Dry weights were then obtained and the tissue transferred to glass scintillation vials for digestion under controlled rehydration (0.5 ml Protosol, New England Nuclear, Boston, Massachusetts, USA; with 0.05 ml distilled water) in an orbital shaker for 18 hours. Scintillation fluid (10 ml, Econoflor, DuPont, Wilmington, Delaware, USA) was then added, the vials vortexed and scintillation counting conducted for five minutes with a wide open window.

Calculation of regional cerebral glucose uptake (rCGlu)

This was achieved by computing the entire ^{14}C DPM/mg (wet weight) contained by the brain as a whole and expressing each sample DPM/mg as a fractional percentage of the whole brain total [27, 28]. This value is thus relative and dimensionless and expresses the fractional accumulation of tracer in each region of interest. This technique adjusts for the large variations in the amount of tracer found in the brains of the different animals and also many of the problems of quantitation characteristic of autoradiographic videodensitometry. Moreover, it has been validated against the latter technique by Meibach et al [27]. Values of rCGlu were computed for each sample within each brain, and the mean value obtained for each treatment to assess the effect of each treatment relative to that of the Sham group. Relative rCGlu (RrCGlu) values were calculated expressing each rCGlu value as a percentage of the parallel Sham treatment for each region of interest.

Statistics

Analysis of variance was conducted on group comparisons, pairwise analysis being made by Student's t-test. Linear regression analysis was performed by the least squares method.

Results

Control of ECF solutes by PD

ECF solutes sampled by the method of low-volume (20 ml) equilibrated peritoneal dialysis (EPD) were obtained before (AM) and following (PM) the daily dialysis period on each of the two days in which PD was conducted in this study, and again on the morning of the third day before sacrifice, during rCGlu estimation. Our data are shown in Figure 3. In undialyzed attrition animals, elevated EPD levels of urea nitrogen (134 ± 4.8 mg/dl), creatinine (2.5 ± 0.9 mg/dl), phosphorus (13.6 ± 4 mg/dl), calcium (5.9 ± 1.7 mg/dl) and potassium (6.4 ± 1.7

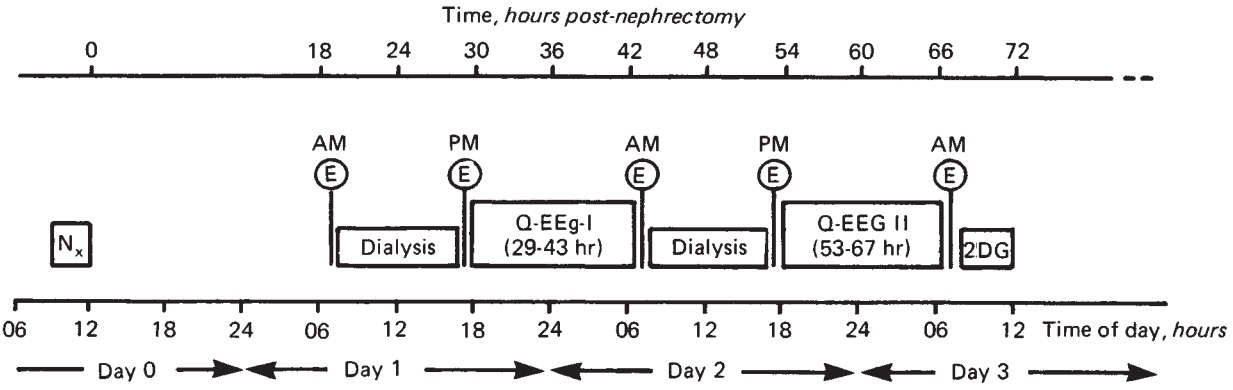

Fig. 2. *Protocol for the treatment of surgically prepared nephrectomized (Nx) or Sham Nx animals.* Dialysis = eight 30 ml, 30 minute exchanges conducted per day except in attrition group rats. E = equilibrated peritoneal dialysis sample obtained for solute analysis (20 ml, TD solution, 60 min). 2DG = animals sacrificed for in vivo regional cerebral glucose uptake studies under the 2-Deoxyglucose protocol.

mEq/l) were apparent by 24 hours following nephrectomy (AM of the following day) compared with Sham operated animals. Abnormally elevated levels of urea N and creatinine continued to rise slightly over time; phosphorus, calcium and potassium remaining at approximately the same elevated level. The regimen of therapeutic dialysis (TD) employing therapeutic dialysate (Table 2) in eight 30-minute exchanges per day appreciably lowered the ECF solute burden as demonstrated by reduced EPD levels of urea nitrogen (69.6 ± 21 mg/dl), creatinine (1.9 ± 0.5 mg/dl), phosphorus (6.8 ± 1.7 mg/dl), calcium (5.7 ± 1.7 mg/dl), and potassium (4.3 ± 1.0 mEq/liter) measured in the PM sample of the first day. Overnight generation of solutes during the time of Q.EEG measurement re-established the previously abnormal levels by the morning of the second day, when the solute-removing effect of TD was again demonstrated. Mock uremic dialysate (M-UD, Table 2) was effective at preventing dialytic extraction of the different solutes with which the solution had been made. EPD solute levels measured in M-UD treated animals were not significantly different from those of the attrition group ($P > 0.05$), in contrast to TD treatment ($P < 0.05$). Phosphate added to therapeutic dialysate (P-UD, Table 2) successfully maintained EPD phosphate levels within the range found in both attrition and M-UD treatments ($P > 0.05$), other EPD-solute levels being comparable with those of the TD group ($P > 0.05$).

Electroencephalographic studies

Preliminary studies showed that within the twenty Hertz frequency spectrum available to our analysis, the delta range (0 to 3 Hz) was highly susceptible to breathing artifacts. The majority of the Q.EEG power, estimated from the compressed spectral array of the fast Fourier transformation resides in the rat as in man, within the ten Hertz range encompassed by theta (3 to 7 H_z) and alpha (7 to 13 Hz) frequency bands which are the components of our index, the theta:alpha ratio (TAR).

The control value (mean ± SEM) of the TAR was thus calculated to be 1.57 ± 0.29 for the entire group of rats, the standard error being 18% of the mean. This variation is compounded of both inter- and intra-animal variation and includes a component, therefore, from each animal's circadian and infradian rhythms over the measuring period (14 hours from each of three days).

Figure 4 illustrates and Figure 5 summarizes (for the 53 to 67 hr Q.EEG measurement interval) the effect of PD treatments on the Q.EEG. No appreciable change from control values was noted in the TAR ratio of EEG powers measured from Sham nephrectomized rats dialyzed using therapeutic dialysates over either of the two Q.EEG measurement periods ($P > 0.05$). Nephrectomy unrelieved by dialysis (attrition treatment) was temporally associated with a linear increase in the TAR ($P < 0.05$ compared with control value). Therapeutic dialysis of nephrectomized animals significantly ($P < 0.001$) reduced TAR values below those in the attrition animals to levels comparable ($P > 0.05$) to those of the sham nephrectomized animals. Dialysis of nephrectomized animals using either M-UD or P-UD solutions (Table 2) produced comparable ($P > 0.05$) levels of TAR that were intermediate between those of the attrition and the TD treated animals ($P < 0.05$).

Relationship between Q.EEG and EPD-solutes

Overnight average concentrations (PM to AM) were calculated for each of the measured solutes so as to obtain a single value representative of each solute's concentration during the period of Q.EEG measurement. Linear least squares regression analysis was then conducted between each animal's overnight average TAR value and its overnight average chemistry values. Small positive correlations were found for urea nitrogen ($r = 0.37$, $P < 0.001$); creatinine ($r = 0.31$, $P < 0.01$); and phosphorus ($r = 0.31$, $P < 0.01$). The correlation between TAR and overnight average calcium concentration ($r = 0.184$) was not statistically significant ($P = 0.09$). These correlations were comparable with values obtained in our earlier work [19]. In the Sham animals, a small positive correlation was found between TAR and urea nitrogen but otherwise no relationship was found. TD animals resembled Sham animals in that the positive correlations associated with the attrition state were not evident. Correlations between TAR and overnight average EPD solute levels was largely negative in the M-UD and P-UD groups.

Regional cerebral glucose uptake studies

At the time of measurement, relative regional cerebral glucose uptake (RrCGlu) values of the animals of the attrition group differed ($P < 0.05$) from those of the Sham group at only three brain sites: hippocampus, geniculate nuclei and the sub-

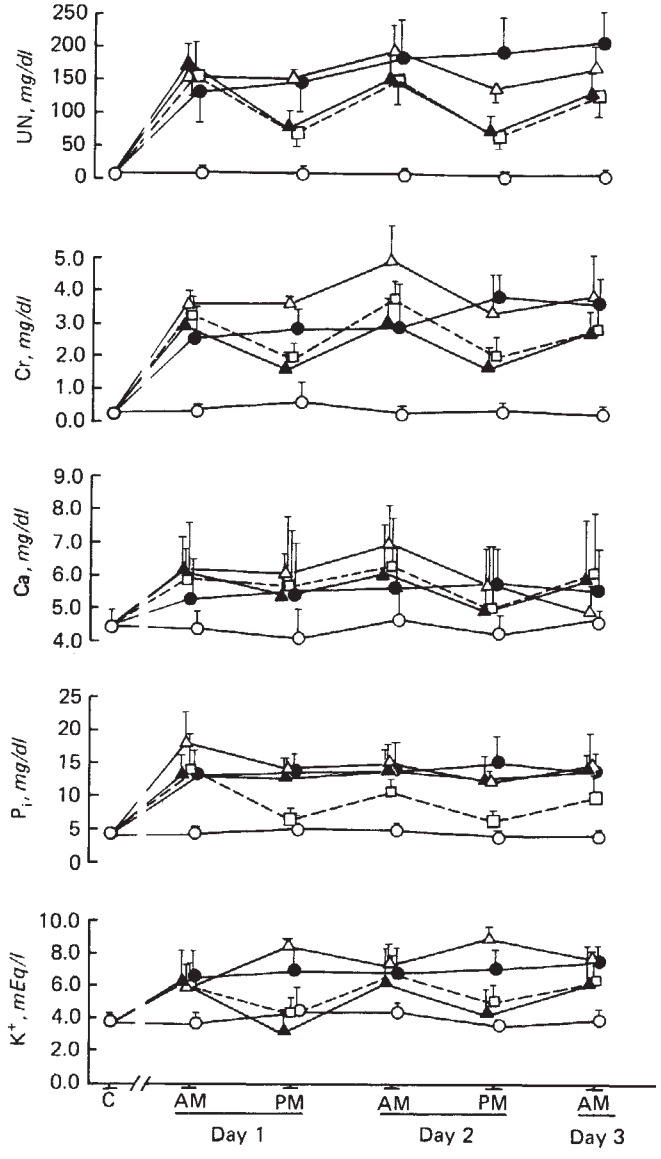

Fig. 3. *Equilibrated peritoneal dialysis (EPD, 20 ml 60 min dwell) levels of solutes sampled throughout the study.* Data are shown for the attrition group (—●—); TD group (--□--); M-UD treatment (—△—); P-UD treatment (—▲—) and Sham dialysis (—○—).

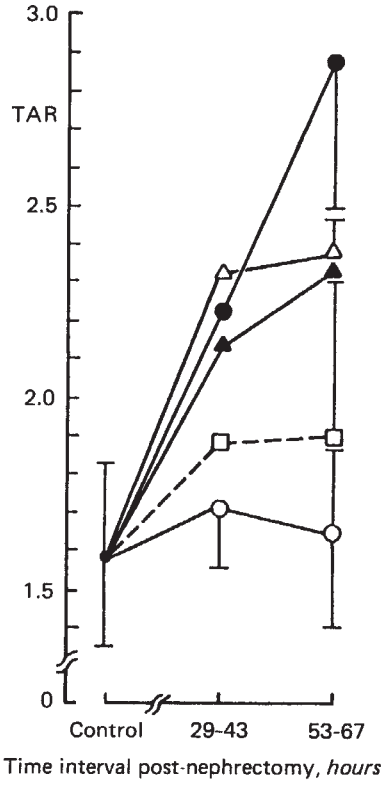

Fig. 4. Average (mean ± SE) theta:alpha ratio (TAR) measured from treated animals during the two 14 hour measuring periods; Q.EEG I, 29 to 43 hours post-nephrectomy and Q.EEG II 53 to 67 hours post-nephrectomy. Protocol is in Figure 2. Symbols are: (●) Attrition; (□) therapeutic dialysis; (△) mock uremic dialysate; (▲) phosphorus dialysis; (○) Sham nephrectomy.

stantia nigra (Fig. 6). The effect of TD treatment on glucose incorporation in these areas is illustrated in Figure 7. At the geniculate bodies the depressed RrCGlu associated with unrelieved nephrectomy was attenuated by a prior history of TD ($P < 0.05$ compared with Sham); but similarly improved values at the hippocampus and substantia nigra did not reach statistical significance.

Discussion

Control of EPD solute concentrations

Abnormal ECF solute burdens accumulate rapidly and inexorably in the totally nephrectomized rat. From Figure 3 it can be seen that the major extent of solute elevation was achieved within the first 24 hours. Thereafter, in the attrition group, EPD-UN appears to increase with a small ($P > 0.05$) linear slope while EPD-creatinine, calcium, phosphorus and potassium levels remained essentially stable at the 24 hour level. As expected, dialyzed, Sham-nephrectomized animals maintained control levels of the measured solutes. Also, as expected, daily TD treatment tended to normalize EPD-solute concentrations while recurrent abnormalities overnight presumably reflected solute generation. The single exception was the rise in total calcium (despite concurrent rise in phosphate), perhaps reflecting the relatively high level of dialysate calcium and possibly increased parathyroid hormone secretion. M-UD treatment generally maintained the EPD solute burden at high levels, sometimes higher than dialysate levels, presumably due to continued solute generation. However, the decrease in PM values on day 2 in spite of the M-UD treatment suggests that endogenous solute generation had slowed, perhaps reflecting decreased solute ingestion (not measured) due to uremic anorexia. As anticipated and intended, P-UD treated nephrectomized animals displayed an EPD solute profile comparable to TD treated rats with the exception of EPD-phosphate levels which were comparable to those of both M-UD and attrition groups ($P > 0.05$).

Association of EPD solutes and Q.EEG

As shown in Figures 4 and 5 the chemical abnormalities in the attrition state are associated with slowing of the Q.EEG back-

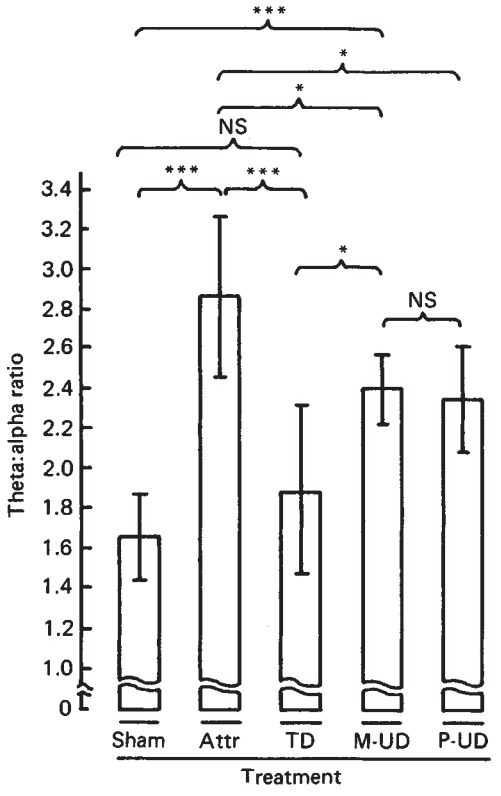

Fig. 5. *Histogram illustrating the TAR (mean ± SE) of the different treatment groups, and the relationships between these during the measuring period Q.EEG II, 53 to 67 hours post-Nx.* Comparisons by Student's *t*-test: $P > 0.05$, NS; $P < 0.05$, *; $P < 0.01$, **; $P < 0.001$ ***.

ground rhythm (increased TAR). Examination of the effect of TD treatment confirms the hypothesis that TAR increase is in some way dialysable-solute-dependent. TD treatment ameliorates both the deranged EPD solute levels and increased TAR of the attrition state. This finding is in agreement with the clinical findings in human and animal studies [3–6, 11–14, 21–23, 29–43] and establishes this model as a clinically relevant bioassay system.

When the removal of ingredient solutes was prevented by M-UD treatment, we nevertheless observed a partial amelioration of the increased TAR due to nephrectomy (Fig. 5). However, the encephalopathy was also not exacerbated. Clearly therefore, some neurotoxic component of the uremic ECF was removed or (alternatively) an indirect adaptive injury was ameliorated by M-UD treatment; this component was not one of the commonly measured solutes with which the M-UD solution had been formulated. We therefore infer: (1) the presence of some other, unmeasured, dialysable neurotoxic substance that is removed (altered) during M-UD dialysis and that produces the slowing effect on the Q.EEG; and (2) that one or more of the M-UD component solutes continues or synergizes with the action of this unknown uremic neurotoxin since the TAR response is incomplete when compared with the effect of TD. Since TD treatment itself reduced elevated TAR values to within the Sham group range, we would suggest that the effect of TD treatment is to reduce the levels of this hypothetical neurotoxin to subtoxic concentrations within our bioassay.

Presumably M-UD treatment was equally efficacious in this regard, yet dialysis with M-UD solutes failed to completely suppress the neurotoxic effects. Since only weak correlations were found between TAR and EPD solutes in the M-UD group, this tends to rule out any direct dose-dependent role for any of these solutes in the neurotoxic effect. On the other hand, increasing levels of the (unknown) neurotoxin could account for the increasing TAR as these EPD levels fell overnight during the time of Q.EEG measurement, probably due to reduced generation of the ingredient solute. It seems plausible to conclude that some component of the M-UD solution interacted with a residual neurotoxic effect to prevent the full ameliorative effect of dialysis (TD) on the Q.EEG. Our findings obtained with the P-UD treatment (Fig. 5) suggest that this component may be phosphorus, or phosphorus-induced. Phosphorus may exert indirect neurotoxic effects via an action on parathyroid hormone. Elevated phosphorus levels are known to stimulate the parathyroid glands to increase PTH production. In an anephric animal such as the present model, the normal negative feedback effect of PTH on phosphorus excretion—stimulation—cannot operate, leading presumably to enhanced PTH levels and further enhancement of its neurotoxicity. Arieff and Massry [54] and also Cooper, Lazarowitz and Arieff [55] have reported—in support of this hypothesis—that abnormal EEG patterns associated with the slowing of the EEG frequencies are correlated with PTH elevation. PTH neurotoxicity may arise from its ability to depress neuronal conduction velocity, an effect most probably engendered via deranged calcium metabolism and increased calcium burden within the nerve, according to the work of Mahoney and Arieff [56]. Goldstein, Chui and Massry [57] have shown that a parathyroid extract reversibly increases the calcium burden of dog nerve. They find, moreover, that depression of conduction velocity is in correlation with the nerve calcium burden.

Solutes other than phosphorus remain to be tested in our assay by means of solute-specific dialysis. Moreover, because generation and retention of other unmeasured solutes presumably proceeded concurrently with those we measured, we are unable to conclude that the measured solutes themselves engendered the associated TAR increases.

The technical and analytic loads of these initial experiments precluded control or examination of such further important variables as nutritional intake (such as, by pair-feeding the animals), dialysate protein loss, nitrogen balance, or the responses of parathyroid or other hormone secretion rates and levels.

ECF solutes, the Q.EEG and brain glucose uptake

Having documented the production by nephrectomy and remission by peritoneal dialysis of uremic encephalopathy in a laboratory model employing conscious ambulatory rats, we next began an initial exploration of possible cerebral mechanisms for these effects. Since glucose is the brain's primary energy source, we hypothesized that glucose metabolism, and perhaps uptake might be altered in this model. Hence measurements of glucose uptake by the 2-Deoxy-D-glucose (2-DG) method [44–48] were studied in attrition, TD and Sham-nephrectomy-dialyzed groups. Because variations of whole-brain uptake between animals were anticipated, and in order to localize cerebral metabolic effects of uremia and dialysis within the

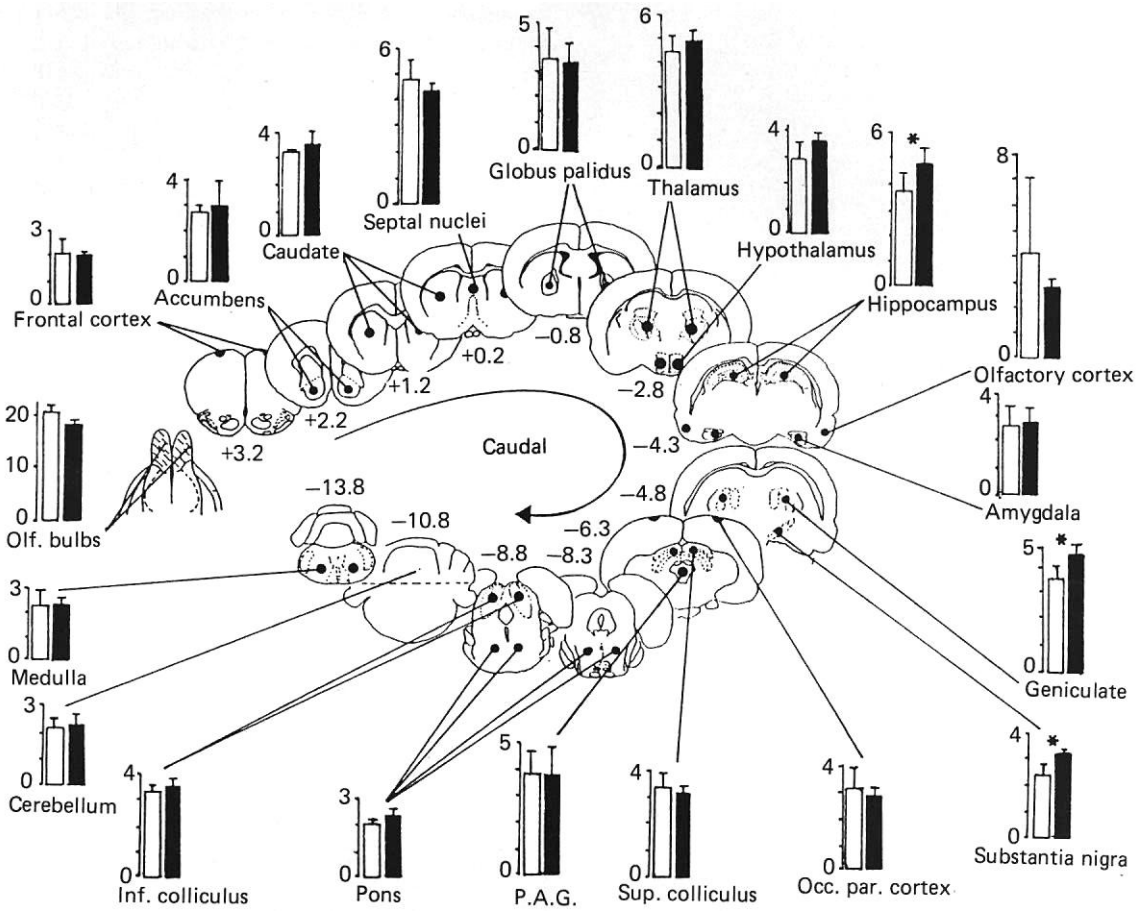

Fig. 6. *Survey of 2DG uptake (rCGlu) in the rat brain in attrition (uremic) animals (open columns) and sham group animals (black columns).*

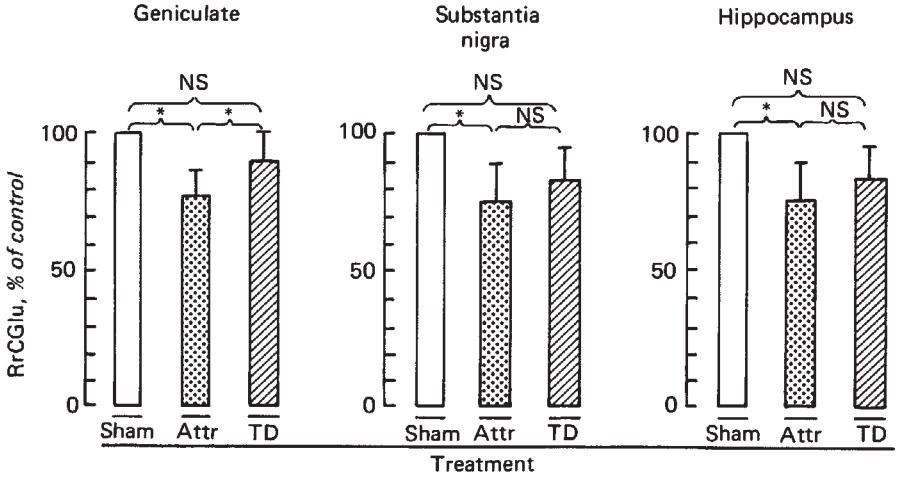

Fig. 7. *Group average (mean ± SEM) regional cerebral glucose uptake values (RrCGlu %) for geniculate nuclei, substantia nigra and hippocampus of animals of the Sham, attrition and TD.* $*P < 0.05$.

brain, methods for direct counting of microdissected brain tissue were chosen [27, 28]. This choice also circumvented problems in the usual techniques of quantifying autoradiographically detected tracer [48]. Precedents for these methods for localization of cerebral metabolic responses to functional activity are known in such applications as olfactory stimulation [46], peripheral nerve stimulation or visual deprivation [47], effects of lysine vasopressin and ACTH fragments [49], the ACTH analogs, alpha-MSH and corticosterone [48] and Naloxone [50]. The neurotoxic effects of aluminum salts have recently been quantified by this technique (Lipman and Tolchard, *Life Science*, in press).

Accordingly 20 brain areas were surveyed and the counts in each were factored by the whole brain uptake in each animal,

then aggregated, to permit comparison of the regional cerebral glucose uptake (rCGlu) in attrition and Sham groups (Fig. 6). While we expected rCGlu deficits in cortical sites and in the thalamic nuclei of origin of the thalamocortical (corticofugal) rhythm generators, no such deficits were found. The neurophysiological source of Q.EEG slowing in this model has thus not been identified, either because the slowed electrical activity does not depend upon reduced glucose uptake, or because the method is too insensitive to detect the small magnitude of changes in these and other brain areas.

Instead, as illustrated in Figure 6, rCGlu was reduced only in the geniculate, substantia nigra and hippocampal areas. When the changes are expressed as *relative* regional cerebral glucose uptake (RrCGlu) as a percent of the Sham group value (Fig. 7), the depressive effect of untreated uremia is again seen in the attrition group. Presumably as a result of prior therapeutic dialysis, glucose uptake in the TD group does not differ statistically from that in the Sham animals, but improvement from attrition levels reaches statistical significance only in the geniculate area. The mechanistic basis for these effects remains to be explored.

These three areas may, however, be functionally relevant to certain functional cerebral deficits in renal failure if not to the Q.EEG slowing. For example, the 'geniculate' tissue sampled in these studies mainly comprised the lateral geniculate nuclei which are the primary neural relays to the visual cortex [52]. A similar metabolic deficit in this area in man may be the biological basis for the deficits in the visual evoked potential latencies observed in human renal failure [34–36, 51]. The substantia nigra, containing the dopaminergic nuclei of the extrapyramidal nigrostriatal tract, is involved in initiation of movement and fine motor control. Deficits here may be responsible for the reduced locomotor activity as well as the tremors typical of uremic encephalopathy in the rat as in man and other species. Finally, the hippocampus is involved in learning and memory, both of which are deficient in encephalopathic uremic patients and in animal models of the condition [39–42, 53].

In conclusion, the present model provides a bioassay with which to investigate the effects and identity of the "uremic neurotoxin(s)" and may find utility in investigations of neuropharmacological effects in the altered uremic state. Our present data suggest that the "unknown dialysable neurotoxin" is peritoneally permeable in this model (that is, has a molecular weight of <15000) and is not identical with any of the commonly measured solutes. Its toxicity is enhanced, however, by uremic levels of one or more of these solutes for which role phosphorus is provisionally a likely candidate.

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